Faculty of Resource Science and Technology

Phytochemical Profiling and Potential Application of Sarawak Liberica Coffee Silverskin

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Liberica coffee is the minority species variety of the *Coffea* family. Internationally, this variety only consisted of 1% of the coffee variety cultivated worldwide. Despite that, this variety is the major species (73%) planted in Malaysia compared to the other varieties. During the roasting of the coffee bean, multiple by-products are produced mainly the coffee silverskin (CS). CS is thin tegument layer outside of the coffee bean, that was generated when treated with high heat intensity. When the coffee production is exponential, this creates an excess of the waste that have multiple potential of application in the industries such as cosmetic, healthcare, and food products. In the recent years, numerous studies had been done on the CS, regarding its beneficial bioactive compounds, as these had shown some promising benefits in multiple industries such as the food production, pharmaceuticals, medicine, and human health. However, most of these studies only focused on the majority variety of the coffee family, thus leaving a knowledge gap on the Liberica CS. As one of the main Liberica cultivators in South-east Asia, the CS variety of the Liberica originate from Sarawak, Malaysia piques the interest in this study. The result showed that, the CS have a high phenolic content in methanol extract and high flavonoid content in ethanol extract, 15.24 ± 0.65 mg GAE/g and 25.14 ± 0.59 mg QE/g, respectively. The DPPH activity was also found to be highest as in the ethanol extract (83.85 ± 1.78%), concurred by the results in the FRAP assay as the highest reduction was also in ethanol extract (11.40 ± 18.57 µmol FSE/g). In terms of the caffeine content, the amount was the highest calculated in the methanol extract with 26.86 ± 5.77 mg/g (1.77 ± 0.38%) of the dry weight of the CS. From the CG-MS employment, the CS was identified to contain 30 bioactive compounds, where four compounds (5-Hydroxymethylfurfural, D-allose, Caffeine, 1,6-Anhydro-β-D-glucofuranose) identified to be the major constituent in the CS, while the rest are in trace

amount. In this study, the CS did not exhibit any antimicrobial effects on both Gram-positive and Gram-negative bacteria. In conclusion, the study shows that Sarawak Liberica CS does contain high beneficial bioactive compounds such as phenolic and flavonoid, while exhibit a significant antioxidant property. The CS may lack in antimicrobial ability, but the high caffeine content of the CS makes it a potential valuable by-product for the industries.

Keywords: Coffee by-product, total phenolic content, total flavonoid content, antioxidant activities, antimicrobial properties, caffeine content

Pemprofilan Fitokimia dan Potensi Kegunaan Kulit Ari Kopi Liberica Sarawak

ABSTRAK

Kopi Liberica adalah spesies minoriti dalam keluarga Coffea. Di peringkat antarabangsa, jenis ini hanya terdiri daripada 1% daripada jenis kopi yang ditanam di seluruh dunia. Walau bagaimanapun, jenis ini merupakan spesies utama (73%) yang ditanam di Malaysia berbanding jenis kopi lain. Semasa proses memanggang biji kopi, pelbagai produk sampingan dihasilkan terutamanya kulit ari (KA). KA adalah lapisan nipis di luar biji kopi, yang tertanggal apabila dipanggang dengan haba yang tinggi. Apabila pemprosesan kopi dilakukan secara besar-besaran, ini akan menghasilkan sisa berlebihan yang mempunyai potensi untuk diaplikasikan dalam pelbagai industri seperti kosmetik, penjagan kesihatan, dan produk makanan. Sejak kebelakangan ini, banyak kajian telah dilakukan ke atas KA, mengenai bahan bioaktifnya yang bermanfaat, dan menunjukkan potensi dalam pelbagai industri seperti penghasilan makanan, ubat-ubatan, perubatan, dan kesihatan manusia. Walau bagaimanapun, kebanyakan kajian ini hanya tertumpu pada kepelbagaian keluarga kopi yang majoriti, sekali gus menghasilkan jurang pengetahuan terhadap KA Liberica. Sebagai salah satu pengeluar Kopi Liberica utama di Asia Tenggara, kepelbagaian KA Liberica Sarawak menjadi fokus dalam kajian ini. Hasil kajian menunjukkan bahawa, KA mempunyai kandungan fenolik yang tinggi dalam ekstrak metanol manakala flavonoid yang tinggi dalam ekstrak etanol, masing-masing 15.24 ± 0.65 mg GAE/g dan 25.14 ± 0.59 mg QE/g. Aktiviti DPPH juga didapati paling tinggi seperti dalam ekstrak etanol (83.85 ± 1.78%), disokong oleh keputusan ujian FRAP kerana pengurangan tertinggi juga dalam ekstrak etanol (11.40 ± 18.57 µmol FSE/g). Dari segi kandungan kafein, yang paling tinggi dikira dalam ekstrak metanol dengan 26.86 ± 5.77 mg/g (1.77 ± 0.38%) daripada berat

bersih KA. Daripada CG-MS, KA telah dikenal pasti mengandungi 30 bahan biokimia, di mana empat sebatian (5-Hydroxymethylfurfural, D-allose, Kafein, 1,6-Anhydro-β-D-glucofuranose) dikenal pasti sebagai juzuk utama dalam KA, manakala selebihnya dalam jumlah yang sedikit. Dalam kajian ini, KA tidak menunjukkan sebarang kesan antimikrob terhadap kedua-dua bakteria Gram-positif dan Gram-negatif. Kesimpulannya, kajian menunjukkan bahawa KA Liberica Sarawak memang mengandungi bahan bioaktif bermanfaat tinggi seperti fenolik dan flavonoid, di samping mempamerkan sifat antioksidan yang ketara. KA mungkin kekurangan keupayaan antimikrob, tetapi kandungan kafein yang tinggi padanya menjadikannya produk sampingan yang berpotensi tinggi sebagai produk berharga dalam pelbagai industri.

Kata kunci: *Produk sampingan kopi, kandungan fenolik, kandungan flavonoid, aktiviti antioksidan, ciri-ciri antimikrobial, kandungan kafein*

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LIST OF ABBREVIATIONS

AAE	Ascorbic acid equivalent
Abs	Absorbance
AST	Antimicrobial Susceptibility Testing
ANOVA	Analysis of variance
ATCC	American Type Culture Collection
±	Approximation
β	Beta
R^2	Correlation coefficient
CLSI	Clinical and Laboratory Standard Institute
CS	Coffee silverskin
CGA	Chlorogenic acid
°C	Celsius
cm	Centimeter
$CHCl_3$	Chloroform
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-piccrylhydrazyl
2,5-DMF	2,5-dimethylfuran
DHT	dihydrotestosterone
eV	Electron volt
E. coli	*Escherichia coli*
FRAP	Ferric Reducing Antioxidant Power

$FeCl_3$	Ferrous chloride
$FeSO_4$	Ferrous sulphate
FSE	Ferrous sulphate equivalent
Fe^{2+}	Ferrous ion
FOS	Fructooligosaccharides
GAE	Gallic acid equivalent
GC	Green chemistry
hr	Hour
HDPE	High-density polyethylene
5-HMF	5-Hydroxymethylfurfural
IC_{50}	Inhibition concentration at 50%
I.D.s	Internal diameters
I	Intermediate
kg	Kilogram
<	Less than
≤	Less or equal
L	Litre
LG	Levoglucosan
MR	Maillard reaction
>	More than
≥	More or equal
µg	Microgram
mg	Milligram
M	Molar
mM	Millimolar

µmol	Micromole
µL	Microlitre
mL	Millilitre
min	Minutes
mm	Millimetre
µm	Micrometre
m/z	Mass-to-charge ratio
MHA	Mueller Hinton Agar
MW	Molecular weight
MF	Molecular formula
MAE	Microwave assisted extraction
MIC	Minimum inhibition concentration
ng	Nanogram
g	Gram
nm	Nanometres
NIST-17	National Institute of Standards and Technology 2017
NB	Nutrient broth
OTA	Ochratoxin A
PLA	Polylactic acid
PBS	Polybutylene succinate
pH	Potential of hydrogen
P	Probability
QE	Quercetin equivalent
RSA	Radical scavenging activities
rpm	Round per minute

R	Resistant
RT	Retention time
RI	Retention index
ROS	Reactive oxygen species
Sdn Bhd	*Sendirian Berhad*
S. aureus	*Staphylococcus aureus*
S	Susceptible
SLE	Solid-Liquid extraction
SWE	Subcritical water extraction
SFE	Supercritical fluid extraction
subsp.	Sub-species
sp.	Species
TPC	Total phenolic content
TFC	Total flavonoid content
TPTZ	2,4,6-Tripyridyl-s-triazine
TE	Trolox equivalent
UV	Ultraviolet
UV-VIS	Ultraviolet visible
UVB	Ultraviolet B
UAE	Ultrasonic assisted extraction
UNIMAS	Universiti Malaysia Sarawak
v/v	Volume-to-volume ratio
w/v	Weight-to-volume ratio
w/w	Weight-to-weight ratio
ZD	Zone diameter

CHAPTER 1

INTRODUCTION

1.1 Study Background

Coffee is one of the most popular beverages worldwide, and its consumption continues to increase every year. In Malaysia alone, the total weight of coffee consumption recently in 2022 was 800,000 of 60 kg bags which bring to total of 48,000 metric tons of coffee (Ahmad, 2022). Quoting from a newspaper report by The Star, the Liberica coffee production in Malaysia was at approximately 325,584 kg in June 2022 (Benjamin, 2022), while the Robusta coffee produced was up to 120,000 kg at the end of 2022 (Statista Research Department). The coffee culture in Malaysia is progressing throughout the year such that the coffee is imported from neighbouring country such as Indonesia, just to satisfy the domestic demands of the consumers. In comparison to Singapore, the third major importer of coffee bean worldwide, Malaysia international trade of shipments was in ratio of 1:6 to Singapore (Volza Grow Global, 2023). Hence, Malaysia is no powerhouse of coffee compared to country such as Brazil, Panama, and Colombia, three main exporters of coffee. Despite that, Malaysia is one of the main Liberica coffee cultivators in the Southeast Asia alongside the Philippines (Anindya, 2021).

As coffee have varieties of species across the globe, Malaysia mainly producing two species which are the Liberica and Robusta variety. Liberica is a rare variety of coffee, and it is grown in small quantity (1%) compared to Arabica and Robusta globally. However, this species accounts for 73% of coffee cultivation in Malaysia, while Robusta coffee comes in second with a 27% coverage, and both are suited for cultivation in Malaysia due to their favourable climate (Ismail et al., 2014). In term of coffee bean production, the Liberica

generated a smaller amount of coffee beans (0.7:10) in kilograms compared to Arabica and Robusta (2:10) in kilograms, by being scarcely available in the market, the Liberica variety serve a higher value in the world coffee market, compared to the other species (Yoong, 2022).

The Sarawak state of Malaysia is well-known for its production of Liberica coffee, a unique minority species of coffee that has adapted to the region's climate and soil conditions. In recent years, researchers have begun to investigate the potential health benefits of Liberica coffee, including its antimicrobial and antioxidant properties. As for the coffee expert and connoisseur, they venture into the exotic taste of the coffee produced from this region, as the taste and aroma of the Liberica coffee were describe as strong, smoky taste, which appreciated by some but not all. Not only that, the Liberica coffee was not a choice of cultivation among the farmers due to the robust and big sized coffee cherries, as this causes inconvenience in post-processing of the coffee. Due to these subjective opinions on the Liberica coffee taste, the species is not the favourite of majority coffee consumer.

In each coffee production, through the dry method of processing, a significant amount of waste would be produced called the coffee silverskin (CS), which accounts for approximately 4% (w/w) of the coffee cherry's weight (Martuscelli et al., 2021). A study by Rodrigues et al. (2015) stated that CS has a high content of cellulose, hemicellulose, and lignin, which makes it a potential source for biofuel production, other than being target of interest in food industries. Despite its potential value as a sustainable biofuel, coffee silverskin is generally considered a waste product and is typically discarded. The further current utilisation and potential future prospect of the CS will be discussed further in this thesis.

1.2 Problem Statement

During the processing of the coffee beans through roasting, approximately 4% (w/w) of the CS would be produced from the coffee cherry. This may seem insignificant on a small production level, however, in the production of 1 tonne of coffee which, approximately 40 kg of CS will be produced, with production of tons of thousands of coffees per year, the CS also produced in excess thus providing us this beneficial by-product to be explore and investigate deeper. In an accumulation of a large amount, the phytotoxic antioxidant such as tannins and polyphenols may emitted pollution towards the soil and water source (Costa et al., 2018).

As far as this study noted, there are no previous reports on Sarawak adapted Liberica coffee silverskin. Previously, a study was done to investigate the antioxidant activity, phenols, flavonoids, antimicrobial, and reducing sugars of the coffee pulp of Sarawak Liberica coffee. The study on Liberica coffee pulp shows promising results as there are high activity of antioxidant observed correlate with the high phenols and flavonoids content (Nillian et al., 2020). This study will contribute to the current knowledge on local Liberica coffee as origin can be a determining factor that contributed to the different in the beneficial compounds level other than roasting, pre and post harvesting process, etcetera.

1.3 Hypotheses

Null hypothesis (H_o): The Liberica coffee silverskin from Sarawak does not possess beneficial bioactive compounds, antioxidant properties, and antimicrobial properties into making it a valuable by-product for various industries.

Alternative hypothesis (H_A): The Liberica coffee silverskin from Sarawak does possess beneficial bioactive compounds, antioxidant properties, and antimicrobial properties making it a valuable by-product for various industries.

1.4 Objectives

This study focal point was to establish a novel look into the potential beneficial properties of Sarawak Liberica coffee silverskin (CS). This was achieved through the following set of objectives:

i. To investigate the effect of ethanol and methanol solvents on the extraction of bioactive compounds in the Sarawak Liberica CS.
ii. To determine the antioxidant activities of Sarawak Liberica CS.
iii. To quantify the caffeine content of the Sarawak Liberica CS.
iv. To determine the antimicrobial properties of Sarawak Liberica CS.